Materials and methods

Sensitization procedures and collection of peritoneal mast cells

Male Sprague-Dawley weighing between 300 and 500 g were sensitized by subcutan injection of 15 mg whole egg white (Sigma Chemical) and killed Bordetella pertussis organisms (5×10^{10}). Four weeks later, mixed peritoneal cells (3 - 5 % mast cells) were harvested in Tris-Gel buffer (composition, mM: Tris, 25; NaCl, 120; KCl, 5; and gelatin 0.01 %, pH 7.6) from peritoneal cavity of rats. The cells were centrifuged and suspended in Tris-Gel CM buffer of the following composition (mM): Tris, 25; NaCl, 120; KCl, 5; $CaCl_2$, 0.6; $MgCl_2$, 1; and 0.01 % gelatin, pH 7.6

Predetermined concentrations of whole egg white (50 µg/ml) and phosphatidylserine (PS, 10 µg/ml, known to enhance allergic histamine secretion) were selected to examine the influence of the drug on allergic histamine release from rat peritoneal mast cells in Tris-Gel CM buffer.

Inhibition of histamine release by drug

Reaction mixtures containing 2×10^6 peritoneal cells (3-5 % mast cells) were preincubated in presence of the drug in polypropylene tubes at 37°C for 15 min. After antigen challenge (final concentration = 50 µg/ml), the cell suspensions were incubated for an additional 30 min at 37°C and then centrifuged at 2000 rpm for 5 min at 4°C. Histamine in the supernatant was measured by the fluormetric method from Shore et al. (1959). (Kopie ist beigefügt). Histamine release, corrected for the spontaneous release, was expressed as a percentage of the total cell content. Total histamine from a separate, duplicate cell suspension was released by boiling for 10 min. Histamine release induced by whole egg white was assayed in the presence and absence of drug.